

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.



⑫

EUROPEAN PATENT SPECIFICATION

⑯ Date of publication of patent specification :
17.03.93 Bulletin 93/11

⑮ Int. Cl.⁵ : A23L 1/305

⑯ Application number : 89111321.9

⑯ Date of filing : 21.06.89

⑯ Amino acid nutrient compositions.

The file contains technical information submitted after the application was filed and not included in this specification

⑯ Priority : 22.06.88 JP 155744/88
26.08.88 JP 212758/88
25.10.88 JP 270558/88
28.10.88 JP 273826/88
07.12.88 JP 310772/88

⑯ Date of publication of application :
27.12.89 Bulletin 89/52

⑯ Publication of the grant of the patent :
17.03.93 Bulletin 93/11

⑯ Designated Contracting States :
DE FR IT

⑯ References cited :
EP-A- 0 182 356
GB-A- 2 071 670
Kirk-Ohmers Encyclopedia of Chemical Technology (1978), p. 405
PATENT ABSTRACTS OF JAPAN, vol. 11, no. 381 (C-464)(2828), 12 December 1987
PATENT ABSTRACTS OF JAPAN, vol. 10, no. 144 (C-349)(2201), 27 May 1986

⑯ References cited :
PATENT ABSTRACTS OF JAPAN, vol. 10, no. 103 (C-340)(2160), 18 April 1986
PATENT ABSTRACTS OF JAPAN, vol. 5, no. 54 (C-50)(726), 15 April 1981

⑯ Proprietor : Roussel Morishita Co., Ltd.
3-8, Doshio-machi 3-chome Chuo-ku
Osaka-shi (JP)
Proprietor : AJINOMOTO CO., INC.
5-8, Kyobashi 1-chome, Chuo-ku
Tokyo 104 (JP)

⑯ Inventor : Ozeki, Kohji
3-22 Kojogaoka Minakuchi-cho Koga-gun
Shiga-ken (JP)
Inventor : Tsukamoto, Yoshitsugu
715 Kawanami Gokasho-cho Kanzaki-gun
Shiga-ken (JP)
Inventor : Yaginuma, Hideya
2030-89 Mikumo Kosei-cho Koga-gun
Shiga-ken (JP)
Inventor : Sato, Makoto
166-40 Harimada-cho
Moriyama-shi Shiga-ken (JP)

⑯ Representative : Strehl, Schübel-Hopf,
Greening
Maximilianstrasse 54 Postfach 22 14 55
W-8000 München 22 (DE)

EP 0 347 890 B1

Note : Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

Description**Field of the Invention:**

5 The present invention provides stabilized and/or dense L-amino acid nutrient compositions. Such stabilized and/or concentrated L-amino acid nutrient compositions may be prepared by using oligopeptides whose molecules contain at least one L-amino acid residue of some specific L-amino acids.

Discussion of the Background:

10 When patients in spite of necessity cannot orally take in, or can take in orally only an insufficient amount of amino acids or proteins, in various diseases or in preoperative or postoperative stage, etc, L-amino acid nutrient compositions whose main ingredients are L-amino acids, e.g., L-amino acid transfusion solutions for intravenous administration (referred to hereinafter as "amino acid transfusion solutions") have been widely utilized for the purpose of nutrient supplementation.

15 In general, however, with the passage of time, amino acid transfusion solutions tend to form degradation products and discolor yellow. Therefore, to prevent discoloration, hitherto stabilizers have been used, such as inorganic salts of sulfurous acid or pyrosulfurous acid, e.g. sodium hydrogensulfite, potassium hydrogensulfite, sodium sulfite, potassium sulfite, sodium pyrosulfite, potassium pyrosulfite, etc. A method for stabilization using sulfurous acid salts of basic amino acids is also disclosed (Japanese Patent Application Laid-Open No. 49-102831). These hydrogensulfites or sulfites are very effective for preventing coloration of amino acid transfusion solutions. Among them, hydrogensulfites are superior.

20 However, it is known that the hydrogensulfites and sulfites react with amino acids. They are very reactive, in particular, with cystine, methionine or tryptophan. For example, these salts react with cystine to cleave the disulfide bond and give cysteine thiosulfate. Furthermore, the salts react with methionine in the presence of oxygen to form methionine sulfoxide. The salts cause an extremely complicated reaction with tryptophan and the major reaction products are highly reactive formylkynurenine, 2,3-dioxyindolealanine, etc. Although, these salts are effective for apparent prevention of coloration of amino acid transfusion solutions they form reaction products which are harmful to the living body. Moreover, the hydrogensulfites or sulfites react with protein to cleave disulfide bonds or bind to protein itself when they are taken into the living body. Furthermore, the salts also react with nucleic acid bases or other compounds in the living body and are known to have a potent mutagenicity.

25 Accordingly, it is not preferred to use the hydrogensulfites or sulfites as stabilizers for amino acid transfusion solutions. Since however, no better stabilizers are known these salts in a trace amount are still in use.

30 It has been now found that the prime cause of instability such as coloration, etc. of amino acid transfusion solutions is attributable to L-tryptophan (Trp) and the coloration is proportional to the concentration of Trp in amino acid transfusion solutions. Trp is one of the essential amino acids. It is known that Trp greatly affects protein synthesis in liver and it has been a demand to increase the Trp concentration in an amino acid transfusion solution. As already stated, however, there have been serious problems in preparation of amino acid

35 transfusion solutions since an increase in the Trp concentration results in increased coloration and the like.

40 A first problem to be solved by the present invention is, under the above-described prior art, to provide stable, L-amino acid-containing nutrient aqueous compositions which contain the Trp ingredient in a desirable amount and which is stable without the use of stabilizers such as hydrogensulfites or sulfites conventionally used.

45 On the other hand, recent researches on the amino acid metabolism under pathological conditions have been promoted and hence the role of the various amino acids under pathological conditions have been made clear. Hence the development of the amino acid transfusion solutions went into two directions, that is, the development of amino acid transfusion solutions according to the respective pathological conditions taking the therapeutic aspect into consideration on one hand and the development of general-purpose amino acid transfusion solutions for correcting the nutrient characteristics relatively common to the various conditions of sickness on the other hand.

50 A nutrient composition comprising higher concentration of amino acids is disclosed in EP-A2-0 182 356. The higher concentrations are achieved by combining free amino acids with oligopeptides. These known nutrient compositions comprise at least a dipeptide or a tripeptide wherein the N-terminal amino acid residue is a glycine residue and at least one dipeptide or tripeptide wherein the N-terminal amino acid residue is selected from alanine, leucine and arginine.

55 Among L-amino acids used for amino acid transfusion solutions, L-tyrosine (Tyr) has been proven essential for liver diseases, uremia, immature infants, newborns, etc. Inter alia, with uremia patients, especially Tyr in-

5 dicates a low level. This is due to that the activity of L-phenylalanine (Phe) hydroxylase is low and thus that the production of Tyr from Phe is inadequate. The decrease in the protein synthesis due to the Tyr deficiency has been recognized to extremely lower the nutrient conditions of the patient. Further, since Tyr is a precursor to catecholamine, it is also indicated that if this is inadequate, various neurosis signs are brought about, and with these patients, Tyr has been gradually taking the position as an essential amino acid. For that reason, it is the present situation that an amino acid transfusion solution in which Tyr is formulated so as to adapt to these pathological conditions has been sought. However, the solubility of Tyr in water is merely 0.045 g/dl at 25°C, and it is difficult to freely formulate a necessary amount thereof as an ingredient for transfusion solution.

10 As regards the formulation of Tyr in transfusion solutions, compositions based on nutrient formulations for healthy humans have heretofore been used, for example, those based on amino acid compositions of human milk or the whole egg according to the report of FAO special committee, 1957. etc. However, as described earlier, in the body of healthy humans Tyr can be synthesized in an adequate amount from Phe, but such synthesis is impossible with certain pathological conditions and thus Tyr is considered as an essential amino acid. Therefore, it is evident that the nutrient formulation for these patients is different from the nutrient formulation 15 for healthy humans.

20 The formulation for pathological conditions taking this into consideration is disclosed in Japanese Patent Application Laid-Open No. 59-16187, where it is indicated that favorable results are obtained when Tyr is contained in the range of 1/12 - 1/17 based on Phe and at a concentration of 0.45 - 0.55 g/l. However, according to the research on the metabolism of the ingested essential amino acid Phe in vivo, it has been discovered that 25 - 70 % of the ingested Phe has been converted into Tyr. It is thought that Phe must be present at a concentration of 5.0 - 10.0 g/l in an ordinary amino acid transfusion solution. Even by simple calculation considering the conversion rate described above, it may be presumed that the part 2.5 - 7.0 g/l of Phe in the formulation must be replaced by Tyr under sick conditions. When this is taken into consideration, the formulation presented in Japanese Patent Application Laid-Open No. 59-16187 has been designed in the range of Tyr solubility, and it seems uncertain that the pathological conditions are well considered. On the other hand, no amino acid transfusion solutions containing such a high concentration of Tyr have been known.

25 In the meantime, several methods for increasing the concentration of Tyr have been proposed. Japanese Patent Application Laid-Open No. 56-8312 discloses a method which utilizes peptides such as L-alanyl-L-tyrosine, L-arginyl-L-tyrosine, L-tyrosyl-L-arginine etc., Japanese Patent Application Laid-Open No. 61-247354 discloses glycyl-L-tyrosine and L-alanyl-L-tyrosine and Japanese Patent Application Laid-Open No. 62-151156 discloses L-aspartyl-L-tyrosine. However, with respect to any of these known preparations it seems uncertain that the necessary formulation of Tyr has been fully studied.

30 Another problem to be solved by the present invention is, under the above-described prior art, to provide an amino acid nutrient transfusion composition of a new formulation which contains sparingly soluble tyrosine in an amount necessary at a ratio achieving the purpose without being subject to the pharmaceutical restrictions and also can exert an excellent nutrient effect to the various considered diseases.

35 As has been discussed earlier, studies on amino acid metabolism under morbid conditions have recently been advanced, and have revealed the roles of various amino acids under pathological conditions. As a result of these studies the trend of developing amino acid transfusion solutions has gone into two different directions: 40 one is to pursue amino acid transfusion solutions used in respective diseases laying a stress on therapy and the other is to pursue all-purpose amino acid transfusion solutions with an attempt to correct nutritive imbalance relatively common to various morbid conditions.

45 With respect to the branched chain L-amino acids (BCAA) L-leucine (Leu), L-isoleucine (Ile) and L-valine (Val) among L-amino acids used in amino acid transfusion solutions, their physiological significance has been clarified over wide areas including application to surgical seizures, hepatic insufficiency, renal insufficiency, septicemia, premature infant, etc. For development of transfusion solutions in either direction described above, attention has been drawn to these amino acids as one of the most important amino acid group. It is known that unlike other amino acids, the branched chain L-amino acids are metabolized mainly in tissues other than liver, and, in particular, Leu has an activity to accelerate synthesis of muscular protein and prevent its decomposition. It is also known that when the branched chain amino acids have been administered to humans in relatively large quantities, their blood concentration does not increase very much and an influence on amino acid distribution in blood is small. Such findings have increased a demand for highly concentrated amino acid transfusion solutions for administration via the central vein in which the ratio of the branched chain amino acids to the total L-amino acids (BCAA/TAA) is increased.

55 However, solubilities of Leu, Ile and Val in water at 25°C are 2.19, 4.12 and 8.85 g/dl, respectively. When other amino acids are co-present, any of the solubilities decreases. For example, a mixture of Leu and Ile in almost equimolar amounts has a solubility of approximately 2.2 g/dl and a mixture of Leu, Ile, Val, L-methionine (Met) and Phe has a solubility of approximately 4.5 g/dl. Thus, when it is wished to raise a concentration of the

branchd chain amino acids, a concentration of other L-amino acids should be extremely reduced so that unbalanced distribution of L-amino acids in blood is caused. Accordingly, its application has been limited to special cases for patients with hepatic encephalosis, etc. That is, it has been difficult to prepare highly concentrated amino acid transfusion solutions containing other L-amino acids in a well-balanced state to be suited for any desired purpose, while increasing a ratio of the branched chain amino acids to the total L-amino acids.

Some proposals have already been made to increase the concentration of the L-amino acid content using water soluble oligopeptides, though the proposals don't pay attention particularly to the branched chain amino acids. For example, in Japanese Patent Application Laid-Open No. 56-140923, there is disclosed a method using at least two oligopeptides containing a glycine residue as the N-terminal. According to this method, however, the proportion of the specific amino acid, glycine (Gly), becomes extremely high to cause imbalanced distribution of L-amino acids in blood, which is not preferable. Furthermore, in Japanese Patent Application Laid-Open No. 61-247354, there is disclosed a method using oligopeptide(s) containing a glycine residue as the N-terminal in combination with oligopeptide(s) containing as the N-terminal a residue from at least one amino acid selected from the group consisting of Ala, L-arginine (Arg) and L-lysine (Lys). However, as is demonstrated in the Laid-Open Applications, an increased concentration of the branched chain amino acids is accompanied by a high concentration of Gly, Ala, Arg or Lys. As a result, it is difficult to provide sitologically preferred compositions.

The present invention aims at providing highly concentrated amino acid transfusion solutions which can exhibit excellent nutrient effect in various diseases. That is, a third problem to be solved by the present invention is, under the above-described prior art, to provide L-amino acid compositions for transfusion in which the ratio of the branched chain amino acid components is increased, other amino acids are formulated to be present in a well balanced state and a high concentration can be achieved without any limitation on preparations.

Furthermore, as has been discussed above, as recent studies on amino acid metabolism under morbid conditions have been advanced, it has been desired to develop amino acid transfusion solutions used for pathologic conditions such as surgical seizures, hepatic insufficiency, renal disorder, septicemia, premature infant, etc. In particular, attention has been drawn to the branched chain L-amino acids (BCAA) metabolized in organs other than liver and by enhancing the proportion of BCAA to the total L-amino acids (TAA), nutrient effects have been increased in pre-operative and post-operative stages. For treatment of some specific disease, there is known Fischer et al's composition applicable to patients with hepatic encephalosis in which the BCAA content is increased while the contents of Met, Phe and Trp are restricted; or the like.

The ratio of BCAA to be incorporated has also been studied, and, as a result, it has been found that an increased ratio of Leu is necessary for exhibiting good effects on sitological parameters. These studies further revealed that when the ratio of BCAA to TAA (BCAA/TAA) is 25 to 60%, good results are obtained. In addition, as has been stated above, even though BCAA is intravenously administered in a relatively large dose, its blood concentration does not increase so that imbalanced distribution of L-amino acids in blood does not occur. Based on these research results, it has been demanded to increase the ratio of BCAA to TAA (BCAA/TAA) and increase the concentration of BCAA more. In particular, as the complete intravenous administration utilizing the central veins has been advanced, the amount of water administered is limited so that a much higher concentration of a transfusion solution has been demanded, as has been also stated above. However, solubilities of Leu, Ile and Val in water at 25°C are 2.19, 4.12 and 8.85 g/dl, respectively. When these amino acids are further mixed with other amino acids, any of the solubilities decreases, as has been already stated. Among them, it is difficult to increase the concentration of Leu, which is sitologically desirable. I.e., there has been a bar against the preparation of dense transfusion solutions having a high concentration of BCAA in compliance with the purpose of use.

Several proposals have already been made, as has been stated above, to increase the concentration of the L-amino acid components in general (Japanese Patent Application Laid-Open No. 56-140923 and Japanese Patent Application Laid-Open No. 61-247354). However, as is demonstrated in the Laid-Open Applications, an increased concentration of BCAA is accompanied by an increased concentration of other L-amino acids, and this is not preferred.

As a result of extensive investigations, the present inventors have found that by mixing a composition mainly composed of BCAA with an ordinary amino acid transfusion solution in a suitable ratio, an amino acid transfusion solution suited for various specific morbid conditions can be readily obtained.

A problem to be solved by the present invention, with respect to the above-described prior art, is to provide BCAA compositions which are used for preparing amino acid transfusion solutions which are free from any preparation restrictions but contain BCAA in a large ratio suited for the purpose in a necessary dose by simply adding such BCAA composition to an ordinary amino acid transfusion solution when administered.

The present invention completed to solve the above problems relates, in one of its aspects, to an aqueous nutrient transfusion solution comprising essential L-amino acids, including L-tryptophan in the form of a di-

peptide, characterized in that

- (a) all of said L-tryptophan is in the form of L-tryptophyl-L-alanine or L-tryptophyl-L-leucine or mixtures thereof and
- (b) that said solution does not comprise a stabilizer.

5 The subject matter of the present invention is further a branched chain L-amino acid composition for amino acid transfusion solutions containing at least one dipeptide whose molecules contain at least one branched chain L-amino acid residue characterized in that the concentration of L-leucine is adjusted to 20 - 100 g/l in the L-amino acid components including the respective component L-amino acids if said dipeptide is calculated as the respective component L-amino acids.

10 Amino acid transfusion solutions and mixtures of L-amino acids which give an amino acid transfusion solution when dissolved in water may be prepared in any conventional ways of preparing amino acid transfusion solutions and mixtures of L-amino acids which give an amino acid transfusion solution when dissolved in water, except that L-tryptophan is present in the form of L-tryptophyl-L-alanine, L-tryptophyl-L-leucine or mixtures thereof.

15 In view of the foregoing findings about the prime cause of the instability of amino acid transfusion solutions, the present inventors have made various investigations and have found that by using Trp in the form of the above defined dipeptide, instability of amino acid transfusion solutions such as discoloration, etc. can be prevented and at the same time, a high concentration of Trp can be achieved.

The peptides can be prepared by conventional peptide synthesis.

20 The amino acid composition in the nutrient transfusion compositions of the present invention may be a new one suited for a desired purpose or may be any conventional one, supposing that the above defined dipeptide used is converted to the component L-amino acids.

25 According to the present invention, L-amino acids and the said dipeptides may be used either in the free form or in the pharmaceutically acceptable salt forms such as metal salts, e.g., with sodium and potassium, mineral acid addition salts, e.g., with hydrochloric and sulfuric acids, and organic acid addition salts, e.g., with acetic and lactic acids.

The nutrient transfusion compositions of the present invention may also contain nutrients other than amino acids, such as electrolytes, trace elements, etc.

30 They are not critical in their concentration, and can have concentrations of known amino acid transfusion solutions. Their pH can be the same as those of known amino acid transfusion solutions, and may usually be in the range of about 4 to about 8. Their pH adjusting agents can be the same ones for known amino acid transfusion solutions.

The present invention will be described in more detail by referring to the examples and the test examples below.

35

EXAMPLE 1

To the amino acid composition shown in Table 1 was added 1.7 g of Trp-Ala. The mixture was dissolved in distilled water for injection by heating to make the whole volume 0.99 liter. After adjusting its pH to about 40 6.5 with an aqueous acetic acid solution, the whole volume was made 1 liter. The solution was filtered through a membrane filter having a pore diameter of 0.45 μ m. The filtrate was filled in a 200 ml glass bottle. After replacing the air with nitrogen gas, the bottle was tight-sealed. A nutrient solution for intravenous administration was then prepared by steam sterilization.

45 The preparation contained 1.3 g/l of Trp and 7.1 g/l of Ala supposing that the dipeptide was converted to the component L-amino acids, i.e., Trp and Ala.

50

55

Table 1: Amino Acid Composition (g)

5	Ile 9.1	Val 14.0	Glu 0.5
10	Leu 12.9	Arg 9.0	Pro 5.0
15	Lys 7.1	His 5.0	Ser 1.7
	Met 4.4	Gly 7.0	Tyr 0.4
	Phe 7.0	Ala 6.5	
	Thr 7.5	Asp 1.0	

20 In the table, Thr, His, Glu, Asp, Pro and Ser represent L-Threonine, L-Histidine, L-Glutamic acid, L-Aspartic acid, L-Proline and L-Serine, respectively.

EXAMPLE 2

25 To the amino acid composition shown in Table 2 was added 3.4 g of Trp-Ala. Subsequently, the mixture was treated in a manner similar to Example 1 to give a transfusion solution.

When the dipeptide was calculated as Trp and Ala, the solution contained 2.5 g/l of Trp and 4.1 g/l of Ala.

Table 2: Amino Acid Composition (g)

30	Ile 7.5	Val 7.5	Cys 0.25
35	Leu 10.0	Arg 2.0	Glu 0.25
40	Lys 5.0	His 2.5	Orn 1.5
	Met 5.0	Gly 2.0	Pro 2.0
	Phe 5.0	Ala 3.0	Ser 1.0
	Thr 2.5	Asp 0.25	Tyr 0.5

45 In the table, Cys and Orn represent L-cysteine and L-ornithine, respectively.

EXAMPLE 3

50 To the amino acid composition shown in Table 3 was added 6.7 g of Trp-Ala. Subsequently, the mixture was treated in a manner similar to Example 1 to give a transfusion solution.

When the dipeptide was calculated as Trp and Ala, the solution contained 5.0 g/l of Trp and 4.7 g/l of Ala.

Table 3: Amino acid Composition (g)

5	Ile	9.1	Val	14.0	Glu	0.5
10	Leu	12.9	Arg	9.0	Pro	5.0
15	Lys	7.1	His	5.0	Ser	1.7
20	Met	4.4	Gly	5.1	Tyr	0.4
25	Phe	7.0	Ala	2.5		
30	Thr	7.5	Asp	1.0		

EXAMPLES 4 to 7

20 The amino acids and dipeptides shown in Table 4 were mixed as is indicated under each example, and the mixtures were treated in a manner similar to Example 1 to give transfusion solutions.

25

30

35

40

45

50

55

Table 4: Amino Acid Compositions (g)

		<u>Example No.</u>			
		<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
10	Ile	7.2	9.1	9.1	7.5
	Leu	11.3	12.9	12.9	10.0
15	Lys	8.1	7.0	7.1	5.0
	Met	11.3	4.4	4.4	5.0
20	Phe	11.3	7.0	7.0	5.0
	Thr	5.2	5.0	7.5	2.5
25	Val	8.3	14.0	14.0	7.5
	Arg	0	9.0	9.0	2.0
30	His	5.7	4.0	5.0	2.5
	Gly	0	3.0	7.0	2.0
35	Ala	0	0	6.5	2.0
	Asp	0	1.0	1.0	0.25
40	Cys	0	0	0.5	0.25
	Glu	0	0.5	0.5	0.25
45	Pro	0	4.0	5.0	2.0
	Ser	0	1.7	1.7	1.0
50	Tyr	0	0.4	0.4	0.5
	Orn	0	0	0	0.5
55	Trp-Ala	3.6	20.1	0	0
	Ala-Trp	0	0	1.7	3.4

TEST EXAMPLE 1

With respect to 0.5% aqueous solution of each of the representative dipeptides, tripeptides and Trp, transmittance after sterilization (105°C, 60 minutes) was measured. The results are shown in Table 5.

In addition, solubility in water was determined. The results are shown in Table 6.

It is noted that any of the peptides are extremely stable as compared to Trp and its solubility is improved.

Table 5: Transmittance after Sterilization (T%, 430 nm)

5	Trp	90.7
10	Trp-Gly	98.5
15	Trp-Ala	99.3
20	Trp-Leu	99.2
25	Ala-Trp	98.1
30	Gly-Trp-Gly	99.2
35	Gly-Trp-Ala	99.1

Table 6: Solubility in Water (g/dl)

20	Trp	1.14
25	Trp-Gly	3.29
30	Trp-Ala	5.20
35	Trp-Leu	3.50
40	Ala-Trp	5.00

TEST EXAMPLE 2

With respect to the-transfusion solutions obtained in Examples 1 to 4 and Comparative Examples 1 to 8, transmittance before and after sterilization (105°C, 80 minutes) was measured, respectively. The results are shown in Tables 7 to 10.

40

45

50

55

Table 7: Transmittance (T%, 430 nm)

5

	<u>Before</u>	<u>After</u>	<u>Difference in</u>
	<u>Sterilization</u>	<u>Sterilization</u>	<u>Transmittance</u>

10

Example 1	99.2	98.9	0.3
------------------	------	------	-----

15

Comparative

Example 1	99.3	97.5	1.8
------------------	------	------	-----

20

Comparative

Example 2	99.2	99.0	0.2
------------------	------	------	-----

25

COMPARATIVE EXAMPLE 1

30

A transfusion solution was prepared by following Example 1 except that Trp and Ala were used instead of Trp-Ala.

35

A transfusion solution was prepared by following comparative Example 1 except that 0.5 g/l of sodium hydrosulfite was additionally added.

40

45

50

55

Table 8: Transmittance (T%, 430 nm)

5

	Before <u>Sterilization</u>	After <u>Sterilization</u>	Difference in <u>Transmittance</u>
--	--------------------------------	-------------------------------	---------------------------------------

10

Example 2	99.6	99.2	0.4
-----------	------	------	-----

15

Comparative

Example 3	99.7	98.3	1.6
-----------	------	------	-----

20

Comparative

Example 4	99.8	99.5	0.4
-----------	------	------	-----

25

COMPARATIVE EXAMPLE 3

30

A transfusion solution was prepared by following Example 2 except that Trp and Ala were used instead of Trp-Ala.

35

COMPARATIVE EXAMPLE 4

A transfusion solution was prepared by following Comparative Example 3 except that 0.5 g/l of sodium hydrosulfite was additionally added.

40

45

50

55

Table 9: Transmittance (T%, 430 nm)

	Before	After	Difference in Sterilization Transmittance
Example 3	98.9	98.5	0.4
Comparative			
Example 5	98.9	95.2	3.7
Comparative			
Example 6	98.9	98.5	0.4

COMPARATIVE EXAMPLE 5

A transfusion solution was prepared by following Example 3 except that Trp and Ala were used instead of Trp-Ala.

COMPARATIVE EXAMPLE 6

A transfusion solution was prepared by following Comparative Example 5 except that 0.5 g/l of sodium hydrosulfite was additionally added.

40

45

50

55

Table 10: Transmittance (T%, 430 nm)

	Before <u>Sterilization</u>	After <u>Sterilization</u>	Difference in <u>Transmittance</u>
Example 4	99.6	99.3	0.3
Comparative			
Example 7	99.7	98.4	1.3
Comparative			
Example 8	99.7	99.4	0.3

COMPARATIVE EXAMPLE 7

30 A transfusion solution was prepared by following Example 4 except that Trp and Ala were used instead of Trp-Ala.

COMPARATIVE EXAMPLE 8

35 A transfusion solution was prepared by following Comparative Example 7 except that 0.5 g/l of sodium hydrosulfite was additionally added.

As described above, it is understood that the compositions of the present invention provide high stability equivalent to the compositions in which sodium hydrosulfite was incorporated.

40 According to the present invention, as is evident from the foregoing examples and test examples, amino acid nutrient transfusion compositions which are sufficiently stable can be provided without using stabilizers such as hydrosulfites or sulfites harmful to the body. Furthermore, the Trp component can be contained in higher concentrations if necessary and desired, and transfusion solutions having new formulations which are suited for various purposes can be provided.

45 The dipeptide and tripeptide used in accordance with the present invention are stable in an aqueous solution and are not colored even after sterilization by heating. Furthermore, solubility in water is also improved.

In fact, the amino acid nutrient transfusion compositions of the present invention containing Trp in the form of dipeptide or tripeptide is not colored without containing any stabilizer therein but is sufficiently stable from an aspect of medical preparations.

50 The dipeptide or tripeptide used in accordance with the present invention is effectively utilized in the living body.

In order to solve another problem described above, the present inventors have made investigations and as a result, have found that branched chain L-amino acid compositions which contain dipeptide(s) of BCAA are provided for amino acid transfusion solution. That is, according to the present invention, the problem in preparations described above can be solved by replacing a part or the whole of Leu, Ile and/or Val with dipeptide(s) whose molecules contain at least one branched chain L-amino acid residue, for example, L-leucyl-L-isoleucine (Leu-Ile), L-isoleucyl-L-leucine (Ile-Leu), L-leucyl-L-valine (Leu-Val), L-valyl-L-isoleucine (Val-Leu), L-isoleucyl-L-valine (Ile-Val) and L-valyl-L-isoleucine (Val-Ile). Thus, amino acid transfusion solutions for intravenous administration which contain the branched chain L-amino acids (BCAA) at a high concentration in a

ratio suited for the intended purpose and have an excellent ratio of Leu, Ile and Val formulated can be prepared with the use of the BCAA compositions of the present invention. The present invention has thus been accomplished on the basis of these findings.

5 That is, the present invention is to provide, in a further embodiment, a branched chain L-amino acid composition containing at least one dipeptide whose molecules contain at least one branched chain L-amino acid residue for amino acid transfusion solutions, which composition is characterized in that the concentration of Leu has been adjusted to 20 - 100 g/l in the L-amino acid components including the respective component L-amino acids if said dipeptide is calculated as the respective component L-amino acids.

10 It is preferred that when the dipeptide is calculated as L-amino acids in a BCAA composition containing at least one dipeptide whose molecules contain at least one branched chain L-amino acid residue for amino acid transfusion solutions, at least the amino acids indicated in Table 11 below are contained in the compositional ranges indicated therein and the weight ratio of Leu, Ile and Val is in the range of 1.6 - 2.4 : 0.8 - 1.2 : 0.8 - 1.2 from the sitological point of view.

15

Table 11

20

Amino Acid	Compositional Range
------------	---------------------

(g/l)

25

Leu	20.0 - 100.0
-----	--------------

30

Ile	10.0 - 50.0
-----	-------------

Val	10.0 - 50.0
-----	-------------

35 The term "when the dipeptide is calculated as L-amino acids" described above means that "when an amount of the dipeptide is calculated as the amounts of the respective L-amino acids formed when the said dipeptide is fully hydrolyzed".

The amino acid composition shown in Table 11 is determined from the preparatory and sitological point of view.

40 Examples of dipeptides containing at least one branched chain L-amino acid residue which can be used in the present invention include Leu-Ile, Ile-Leu, Leu-Val, Val-Leu, Ile-Val, Val-Ile, as has been mentioned above.

In a BCAA composition of the present invention, the ratio of the BCAA in the free state and the BCAA in the dipeptide(s) form is 0 - 100 : 4 - 300.

45 The composition of the present invention cannot be used as an amino acid transfusion solution as it is. However, as will be shown below, amino acid transfusion solutions suitable for particular morbid conditions can readily be prepared by mixing the composition of the present invention with other amino acid compositions in an appropriate ratio.

50 Desired amino acid transfusion solutions can be prepared conventionally in a manner similar to known amino acid transfusion solutions except a composition of the present invention is used. Any pH value can be taken but preferred is between 4.5 and 8.0 from the physiological point of view.

Furthermore, nutrient substances such as sugars, vitamins, minerals, etc. may also be incorporated utilizing known techniques. Even when reducing sugars are formulated in the amino acid transfusion compositions, it is difficult to cause the Maillard reaction and in this sense, the compositions are advantageous.

55 The present invention will be described in more detail by referring to the examples and preparation examples below.

EXAMPLE 8

5 The branched chain amino acids and dipeptides containing the branched chain amino acid residues shown in Table 12 below were dissolved in distilled water for injection by heating to make the whole volume 0.99 liter. After adjusting its pH to 6.0 to 7.0 with an aqueous acetic acid solution, the whole volume was made 1 liter. The solution was filtered through a membrane filter having a pore diameter of 0.45 µm. The filtrate was filled in a 200 ml glass bottle. After replacing the air with nitrogen gas, the bottle was tight-sealed. This was sterilized with steam under high pressure to prepare a BCAA composition, which was supposed to be used in preparing amino acid transfusion solutions. The composition of this example contained 30.0 g/l of Ile, 50.0 g/l of Leu and 10 25.0 g/l of Val.

Table 12

15

20

25

30

35

40

45

50

55

Amino Acid and Dipeptide	Amount Used (g/l)
Ile	10.0
Leu	10.0
Val	7.1
Leu-Ile	37.3
Leu-Val	35.1

In Examples 9 - 23, BCAA compositions to be used for amino acid transfusion solutions were prepared in a conventional manner using dipeptides containing branched chain L-amino acid residues and/or branched chain L-amino acids indicated in Tables 13 - 27, respectively.

EXAMPLE 9Table 13

5

10

15

20

25

30

35

40

45

50

55

Amino Acid and Dipeptide	Amount Used (g/l)
Ile	5.0
Leu	10.0
Val	12.1
Ile-Leu	37.3
Val-Leu	35.1

The composition of this example contained 25.0 g/l of Ile, 50.0 g/l of Leu and 30.0 g/l of Val.

EXAMPLE 10Table 14

Amino Acid and Dipeptide	Amount Used (g/l)
Ile	5.0
Leu	10.0
Val	7.1
Leu-Ile	37.3
Val-Leu	35.1

The composition of this example contained 25.0 g/l of Ile, 50.0 g/l of Leu and 25.0 g/l of Val.

EXAMPLE 11Table 15

5

10

15

20

25

30

35

40

45

50

	Amino Acid and Dipeptide	Amount Used (g/l)
	Ile	10.0
	Leu	10.0
	Val	12.1
	Ile-Leu	74.5
	Leu-Val	35.1

The composition of this example contained 50.0 g/l of Ile, 70.0 g/l of Leu and 30.0 g/l of Val.

EXAMPLE 12Table 16

	Amino Acid and Dipeptide	Amount Used (g/l)
	Ile	10.0
	Leu	20.0
	Val	20.0
	Leu-Ile	37.3

The composition of this example contained 30.0 g/l of Ile, 40.0 g/l of Leu and 20.0 g/l of Val.

55

EXAMPLE 13Table 17

5

10

Amino Acid and Dipeptide	Amount Used (g/l)
Leu	20.0
Val	25.0
Leu-Ile	46.6

20

The composition of this example contained 25.0 g/l of Ile, 45.0 g/l of Leu and 25.0 g/l of Val.

EXAMPLE 14

25

Table 18

30

35

40

Amino Acid and Dipeptide	Amount Used (g/l)
Val	2.1
Ile-Leu	37.3
Leu-Val	35.1

The composition of this example contained 20.0 g/l of Ile, 40.0 g/l of Leu and 20.0 g/l of Val.

45

50

55

EXAMPLE 15Table 19

5

10

	Amino Acid and Dipeptide	Amount Used (g/l)
	Val	0.1
15	Leu-Ile	27.9
	Val-Leu	35.1

20

The composition of this example contained 15.0 g/l of Ile, 35.0 g/l of Leu and 18.0 g/l of Val.

EXAMPLE 16

25

Table 20

30

35

40

	Amino Acid and Dipeptide	Amount Used (g/l)
	Leu	15.0
	Val	15.0
	Ile-Leu	27.9

The composition of this example contained 15.0 g/l of Ile, 30.0 g/l of Leu and 15.0 g/l of Val.

45

50

55

EXAMPLE 17

5

Table 21

10

	Amino Acid and Dipeptide	Amount Used (g/l)
	Leu	10.0
15	Val	10.0
	Ile-Leu	18.6

20

The composition of this example contained 10.0 g/l of Ile, 20.0 g/l of Leu and 10.0 g/l of Val.

EXAMPLE 18

25

Table 22

30

	Amino Acid and Dipeptide	Amount Used (g/l)
	Leu	20.0
35	Val	15.5
	Leu-Ile	37.3
	Ile-Val	8.8

45

The composition of this example contained 25.0 g/l of Ile, 40.0 g/l of Leu and 20.0 g/l of Val.

50

55

EXAMPLE 19Table 23

5

10

15

20

25

30

35

40

45

50

55

Amino Acid and Dipeptide	Amount Used (g/l)
Leu	20.0
Val	1.1
Val-Ile	17.6

The composition of this example contained 10.0 g/l of Ile, 20.0 g/l of Leu and 10.0 g/l of Val.

EXAMPLE 20Table 24

Amino Acid and Dipeptide	Amount Used (g/l)
Leu	20.0
Val	1.6
Ile-Val	26.3

The composition of this example contained 15.0 g/l of Ile, 20.0 g/l of Leu and 15.0 g/l of Val.

EXAMPLE 21Table 25

5

10

15

20

25

30

35

40

45

50

55

	Amino Acid and Dipeptide	Amount Used (g/l)
	Ile	10.0
	Leu	10.0
	Val	12.1
	Leu-Ile	74.5
	Val-Leu	35.1

The composition of this example contained 50.0 g/l of Ile, 70.0 g/l of Leu and 30.0 g/l of Val.

EXAMPLE 22Table 26

	Amino Acid and Dipeptide	Amount Used (g/l)
	Leu-Ile	37.3
	Leu-Val	35.1

The composition of this example contained 20.0 g/l of Ile, 40.0 g/l of Leu and 17.9 g/l of Val.

EXAMPLE 23Table 27

5

10

15

20

30

35

40

45

50

55

	Amino Acid and Dipeptide	Amount Used (g/l)
	Leu-Ile	74.5
	Leu-Val	70.2

The composition of this example contained 40.0 g/l of Ile, 80.0 g/l of Leu and 35.8 g/l of Val.

PREPARATION EXAMPLES 1 - 5

Portions of the BCAA composition obtained in Example 10 were mixed with a commercially available amino acid transfusion solution in various ratios in a conventional manner. Thus, amino acid transfusion solutions 1 - 5 were prepared.

The compositions of the amino acids in the transfusion solutions thus prepared are shown in Table 28.

Table 28 : Composition of Amino Acids after Mixing

5

10

15

20

25

30

35

40

45

50

Preparation Example No.	1	2	3	4	5
Mixing Ratio	0.5:1	1:1	0.1:1	0.1:1	0.1:1
Ile	11.8	15.0	7.1	11.5	10.5
Leu	24.2	30.7	15.4	19.3	16.3
Lys	8.1	6.1	10.9	13.8	6.5
Met	2.3	1.7	3.1	13.4	4.0
Phe	6.1	4.6	8.2	13.4	6.4
Thr	4.2	3.2	5.7	6.1	6.8
Trp	0.85	0.63	1.1	3.0	1.2
Val	11.1	14.5	6.2	12.1	15.0
Ala	4.0	3.0	5.5	6.7	6.5
Arg	5.1	3.9	7.0	-	8.2
Asp	2.5	1.9	3.3	-	0.91
Cys	0.65	0.49	0.88	-	-
Glu	4.2	3.2	5.7	-	0.45
His	3.9	2.9	5.3	-	4.5
Pro	2.1	1.6	2.9	-	4.5
Ser	1.4	1.1	1.9	-	1.5
Tyr	0.22	0.17	0.31	-	0.36
Gly	7.0	5.3	9.4	-	6.4

Preparations Nos. 1, 2 and 5 in the table above were obtained by mixing the BCAA composition with a commercially available amino acid transfusion solution in ratios of 0.5:1, 1:1 and 0.1:1, in which preparations the content of branched chain L-amino acids was relatively high and the glycine content was relatively low since glycine tends to cause hyperammonemia. These preparations are suitably administered to patients in the post-operative stage or patients without any abnormality in amino acid metabolism.

Preparation No. 3 described above was obtained by mixing the BCAA composition with the commercially available amino acid transfusion solution in a ratio of 0.1 : 1, in which preparation branched chain amino acids metabolized in organs other than liver were contained in large quantities. The preparation can improve nutritional conditions of patients with hepatic disorder.

5 Preparation No. 4 described above was obtained by mixing the BCAA composition with the same amino acid transfusion solution in a ratio of 0.1 : 1, in which preparation the content of non-essential L-amino acids was reduced so as to re-use blood urea and ammonia for synthesis of L-amino acids. The preparation is suited for administration to patients with renal disorder.

10 Thus, it is understood that by appropriately changing the mixing ratio as described above, amino acid transfusion solutions having desired compositions can readily be obtained by using BCAA compositions of the present invention.

15 As is evident especially from the foregoing examples and preparation examples, the BCAA composition of the present invention contains branched chain L-amino acids in a high concentration in a suitable ratio and is useful as a premixing preparation for preparing amino acid transfusion solutions for respective morbid conditions which are required to administer highly dense branched chain L-amino acids. In other words, if a conventional amino acid transfusion solution is mixed with a BCAA composition of the present invention in an appropriate ratio, then amino acid transfusion solutions can readily be prepared which solutions are suited for respective specific diseases.

20 According to the present invention, since branched chain L-amino acids are in the form of dipeptides, compositions for amino acid transfusion solutions can be provided in a ratio suited for the intended purposes, without any preparatory restrictions. The composition of the present invention is useful as a composition which can be formulated in amino acid transfusion solutions used for respective morbid conditions.

25 The dipeptides in accordance with the present invention are effectively utilized in the living body.

25

Claims

1. An aqueous nutrient transfusion solution comprising essential L-amino acids, including L-tryptophan in the form of a dipeptide, characterized in that
 - 30 (a) all of said L-tryptophan is in the form of L-tryptophyl-L-alanine or L-tryptophyl-L-leucine or mixtures thereof and
 - (b) that said solution does not comprise a stabilizer.
2. An aqueous nutrient transfusion solution according to claim 1, which in addition to constituent (a) comprises at least one amino acid selected from the group consisting of L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-valine, L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glutamic acid, glycine, L-histidine, L-ornithine, L-proline, L-serine and L-tyrosine.
3. A branched chain L-amino acid composition for amino acid transfusion solutions containing at least one dipeptide whose molecules contain at least one branched chain L-amino acid residue characterized in that the concentration of L-leucine is adjusted to 20 - 100 g/l in the L-amino acid components including the respective component L-amino acids if said dipeptide is calculated as the respective component L-amino acids.

45

Patentansprüche

1. Wässrige Nährtransfusionslösung umfassend essentielle L-Aminosäuren, einschließlich L-Tryptophan in der Form eines Dipeptids, dadurch gekennzeichnet, daß
 - 50 (a) das gesamte L-Tryptophan in der Form von L-Tryptophyl-L-alanin, oder L-Tryptophyl-L-leucin oder Mischungen davon vorliegt, und
 - (b) die Lösung keinen Stabilisator enthält.
2. Wässrige Nährtransfusionslösung nach Anspruch 1, welche neben Bestandteil (a) zusätzlich wenigstens eine Aminosäure umfasst, ausgewählt aus der Gruppe bestehend aus L-Isoleucin, L-Leucin, L-Lysin, L-Methionin, L-Phenylalanin, L-Threonin, L-Valin, L-Alanin, L-Arginin, L-Asparaginsäure, L-Cystein, L-Glutaminsäure, Glycin, L-Histidin, L-Ornithin, L-Prolin, L-Serin und L-Tyrosin.

3. Zusammensetzung auf Basis einer verzweigtkettigen L-Aminosäure für Aminosäuretransfusionslösungen, enthaltend wenigstens ein Dipeptid, dessen Moleküle wenigstens einen verzweigtkettigen L-Aminosäurerest enthalten, dadurch gekennzeichnet, daß die Konzentration an L-Leucin in den L-Aminosäurekomponenten auf 20 - 100 g/l, einschließlich der entsprechenden Komponente der L-Aminosäure eingestellt wird, wenn das Dipeptid als die jeweils als Komponenten vorliegenden L-Aminosäuren berechnet wird.

Revendications

10 1. Une solution de transfusion nutritive aqueuse comprenant des L-aminoacides essentiels, incluant le L-tryptophane sous la forme d'un dipeptide, caractérisée en ce que :
(a) la totalité du L-tryptophane est sous la forme de L-tryptophyl-L-alanine ou de L-tryptophyl-L-leucine ou d'un mélange des deux et
(b) que ladite solution ne comprend pas un stabilisant.

15 2. Une solution de transfusion nutritive aqueuse selon la revendication 1 qui en plus du constituant (a) comprend au moins un aminoacide choisi dans le groupe comprenant les suivants : L-isoleucine, L-leucine, L-lysine, L-méthionine, L-phénylalanine, L-thréonine, L-valine, L-alanine, L-arginine, acide L-aspartique, L-cystéine, acide L-glutamique, glycine, L-histidine, L-ornithine, L-proline, L-sérine et L-tyrosine.

20 3. Une composition de L-aminoacides à chaîne ramifiée pour solutions de transfusion d'aminoacides contenant au moins un dipeptide dont les molécules contiennent au moins un résidu de L-aminoacides à chaîne ramifiée, caractérisée en ce que la concentration en L-leucine est ajustée à 20-100 g/l dans les composants L-aminoacides incluant les L-aminoacides composants respectifs lorsque le dipeptide est calculé comme L-aminoacides composants respectifs.

30

35

40

45

50

55